

REMARKS

I. Amendments

Claims 1-4, 10, 16, 30, 36 and 37 were withdrawn, claims 5-9, 11-15, 17-19, 31-35, 38, 51 and 52 were canceled, claim 39 has been amended, and claims 53-57 have been added. Upon entry of the amendment, claims 39-50 and 53-57 will be pending. The amendment does not constitute new matter and is supported throughout the specification, for example, page 7, lines 11-14; Figure 2; and in the claims as originally filed.

II. Rejections

A. *Rejections under 35 U.S.C. § 101*

Claims 39-52 stand rejected as the claimed invention allegedly is not supported by either a specific or substantial asserted utility or a well-established utility. The claims as amended are drawn to a transgenic mouse whose genome comprises a homozygous disruption in a gene encoding mCAR2, wherein as a result of the disruption, the transgenic mouse lacks production of functional protein encoded by said gene and exhibits, relative to a wild-type mouse, impaired coordination or balance, a spleen abnormality, a thymus abnormality or a lymph node abnormality. The Examiner essentially argues that: (i) studying the mouse to determine the function of a gene is not in and of itself a substantial utility (page 8); and (ii) none of the phenotypes correlate to a useful phenotype because the phenotypes described are not specific to a disease and are not linked to a disruption in the human equivalent of SQ ID NO:1 (page 4).

Applicant respectfully disagrees.

According to 35 U.S.C. § 101, “[w]hoever invents . . . any new and useful . . . composition of matter may obtain a patent therefore. . . .”

Under the Patent Office’s Utility Requirement Guidelines:

If at any time during the examination, it becomes readily apparent that the claimed invention has a well-established utility, do not impose a rejection based on lack of utility. An invention has a well-established utility if (i) a person of ordinary skill in the art would immediately appreciate why the invention is useful based on the characteristics of the invention (e.g., properties or applications of a product or process), and (ii) the utility is specific, substantial, and credible.

...

If the applicant has asserted that the claimed invention is useful for any particular practical purpose (i.e., it has a “specific and substantial utility”) and the assertion would be considered credible by a person of ordinary skill in the art, do not impose a rejection based on lack of utility.

(emphasis added)(MPEP § 2107, II (A)(3); II (B)(1)). Thus, according to Patent Office guidelines, a rejection for lack of utility may not be imposed where an invention has either a well-established utility or is useful for a particular practical purpose. The present invention satisfies either standard.

The present invention has a well-established utility since a person of ordinary skill in the art “would immediately appreciate why” knockout mice are useful. As a general principle, any knockout mouse has the inherent and well-established utility of defining the function and role of the disrupted target gene, regardless of whether the inventor has described any specific phenotypes, characterizations or properties of the knockout mouse. The sequencing of the human genome has produced countless genes whose function has yet to be determined. According to the National Institute of Health, knockout mice represent a critical tool in studying gene function:

Over the past century, the mouse has developed into the premier mammalian model system for genetic research. Scientists from a wide range of biomedical fields have gravitated to the mouse because of its close genetic and physiological similarities to humans, as well as the ease with which its genome can be manipulated and analyzed.

...
In recent decades, researchers have utilized an array of innovative genetic technologies to produce custom-made mouse models for a wide array of specific diseases, as well as to study the function of targeted genes. One of the most important advances has been the ability to create transgenic mice, in which a new gene is inserted into the animal's germline. Even more powerful approaches, dependent on homologous recombination, have permitted the development of tools to "knock out" genes, which involves replacing existing genes with altered versions; or to "knock in" genes, which involves altering a mouse gene in its natural location. To preserve these extremely valuable strains of mice and to assist in the propagation of strains with poor reproduction, researchers have taken advantage of state-of-the-art reproductive technologies, including cryopreservation of embryos, in vitro fertilization and ovary transplantation.

(<http://www.genome.gov/pfv.cfm?pageid=10005834>) (emphasis added). Thus, the knockout mouse has been accepted as by the NIH as the premier model for determining gene function, a utility that is specific, substantial and credible.

Knockout mice are so well accepted as tools for determining gene function that the director of the NIH Chemical Genomics Center of the National Human Genome Research Institute (among others, including Capecchi, Bradley, Joyner, Nagy and Skarnes) has proposed creating knockout mice for all mouse genes:

Now that the human and mouse genome sequences are known, attention has turned to elucidating gene function and identifying gene products that might have therapeutic value. The laboratory mouse (*Mus musculus*) has had a prominent role in the study of human disease mechanisms throughout the rich, 100-year history of classical mouse genetics, exemplified by the lessons learned from naturally occurring mutants such as agouti, reeler and obese. The large-scale production and analysis of induced genetic mutations in worms, flies, zebrafish and mice have greatly accelerated the understanding of gene function in these organisms. Among the model organisms, the mouse offers particular advantages for the study of human biology and disease: (i) the mouse is a mammal, and its development, body plan, physiology, behavior and diseases have much in common with those of humans; (ii) almost all (99%) mouse genes have homologs in humans; and (iii) the mouse genome supports targeted mutagenesis in specific genes by homologous recombination in embryonic stem (ES) cells, allowing genes to be altered efficiently and precisely.

...

A coordinated project to systematically knock out all mouse genes is likely to be of enormous benefit to the research community, given the demonstrated power of knockout mice to elucidate gene function, the frequency of unpredicted phenotypes in knockout mice, the potential economies of scale in an organized and carefully planned project, and the high cost and lack of availability of knockout mice being made in current efforts.

(Austin et al., *Nature Genetics* (2004) 36(9):921-24, 921)(emphasis added)(copy attached).

With respect to new claims 53-57, drawn to transgenic mice having a null allele, the following comments from Austin are relevant:

Null-reporter alleles should be created

The project should generate alleles that are as uniform as possible, to allow efficient production and comparison of mouse phenotypes. The alleles should achieve a balance of utility, flexibility, throughput and cost. A null allele is an indispensable starting point for studying the function of every gene. Inserting a reporter gene (e.g., P-galactosidase or green fluorescent protein) allows a rapid assessment of which cell types normally

support the expression of that gene.

(p. 922)(emphasis added).

According to the Molecular Biology of the Cell (Albert, 4th ed., Garland Science (2002)), one of the leading textbooks in the field of molecular biology:

Extensive collaborative efforts are underway to generate comprehensive libraries of mutation in several model organisms including . . . the mouse. The ultimate goal in each case is to produce a collection of mutant strains in which every gene in the organism has either been systematically deleted, or altered such that it can be conditionally disrupted. Collections of this type will provide an invaluable tool for investigating gene function on a genomic scale.

(p. 543)(emphasis added).

According to Genes VII (Lewin, Oxford University Press (2000)), another well respected textbook in the field of genetics:

The converse of the introduction of new genes is the ability to disrupt specific endogenous genes. Additional DNA can be introduced within a gene to prevent its expression and to generate a null allele. Breeding from an animal with a null allele can generate a homozygous “knockout”, which has no active copy of the gene. This is a powerful method to investigate directly the importance and function of the gene.

(p. 508)(emphasis added).

Knockout mice may be appropriately analogized to other research tools, with respect to which the Patent Office has commented:

Some confusion can result when one attempts to label certain types of inventions as not being capable of having a specific and substantial utility based on the setting in which the invention is to be used. One example is inventions to be used in a research or laboratory setting. Many research tools such as gas chromatographs, screening assays, and nucleotide sequencing techniques have a clear, specific and unquestionable utility (e.g., they are useful in analyzing compounds). An assessment that focuses on whether an invention is useful only in a research setting thus does not address whether the invention is in fact “useful” in a patent sense. Instead, Office personnel must distinguish between inventions that have a specifically identified substantial utility and inventions whose asserted utility requires further research to identify or reasonably confirm. Labels such as “research tool,” “intermediate” or “for research purposes” are not helpful in determining if an applicant has identified a specific and substantial utility for the invention.

(MPEP § 2107.01, I). The use of knockout mice as research tools is recognized by those skilled in the art. For example, according to Crabbe *et al.* (Science (1999) 284:1670-1672) a reference cited by the Examiner, “[t]argeted and chemically induced mutations in mice are valuable tools in biomedical research.” As with gas chromatographs, screening assays and nucleotide sequencing techniques, knockout mice have a clear, specific and unquestionable utility (e.g., they are useful in analyzing gene function).

The role of knockout mice in studying the function of orphan nuclear hormone receptors is well-established. For example, Luo *et. al.* (*J Steroid Biochem Mol Biol.* 1999, 69(1-6):13-8) generated a transgenic mouse having a disruption in SF-1, an orphan nuclear hormone receptor:

Steroidogenic factor 1 (SF-1), an orphan nuclear receptor, initially was isolated as a key regulator of the tissue-specific expression of the cytochrome P450 steroid hydroxylases. Thereafter, analyses of sites of SF-1 expression during mouse embryological development hinted at considerably expanded roles for SF-1, roles that were strikingly confirmed through the analyses of SF-1 knockout mice. These SF-1 knockout mice exhibited adrenal and gonadal agenesis, associated with male-to-female sex reversal of their internal and external genitalia and death from adrenocortical insufficiency. These findings showed unequivocally that SF-1 is essential for the embryonic survival of the primary steroidogenic organs. SF-1 knockout mice also had impaired pituitary expression of gonadotropins and agenesis of the ventromedial hypothalamic nucleus (VMH), establishing that SF-1 regulates reproductive function at all three levels of the hypothalamic-pituitary gonadal axis.

(abstract). By way of further example, Choi *et al.* (*Genes Dev.* (1994) 8(20):2466-77) produced a transgenic mouse have a deficiency in HNF-4, another orphan nuclear hormone receptor:

Expression of HNF-4, a transcription factor in the steroid hormone receptor superfamily, is detected only in the visceral endoderm of mouse embryos during gastrulation and is expressed in certain embryonic tissues from 8.5 days of gestation. To examine the role of HNF-4 during embryonic development, we disrupted the gene in embryonic stem cells and found that the homozygous loss of functional HNF-4 protein was an embryonic lethal. Cell death was evident in the embryonic ectoderm at 6.5 days when these cells normally initiate gastrulation. As assessed by expression of Brachyury and HNF-3 beta, primitive streak formation and initial differentiation of mesoderm do occur, but with a delay of approximately 24 h. Development of embryonic structures is severely impaired. These results demonstrate that the expression of HNF-4 in the visceral endoderm is essential for embryonic ectoderm survival and normal gastrulation.

(abstract). Both of these examples illustrate that knockout mice are widely used to study gene function.

Commercial use and acceptance is one important indication that the utility of an invention has been recognized by one of skill in the art (“A patent system must be related to the world of commerce rather than to the realm of philosophy.” *Brenner v Manson*, 383 U.S. 519, 148 U.S.P.Q. 689, 696 (1966)). Commercial use of the knockout mice produced by Assignee Deltagen has been clearly established. Deltagen has created a database comprising characteristics derived from approximately 750 lines of knockout mice. Three of the largest pharmaceutical companies in the world, Merck, Pfizer and GSK, have subscribed to the database and requested access to the lines of mice for the purpose of studying gene function. In fact, no fewer than four (4) commercial and academic institutions have ordered the presently claimed mCAR2 knockout mouse. This commercial acceptance more than satisfies the practical utility requirement of section 101 as it cannot be reasonably argued that a claimed invention which is actually being commercially used has no use (see, for example, Phillips Petroleum Co. v. U.S. Steel Corp., 673 F. Supp. 1278, 6 U.S.P.Q.2d 1065, 1104 (D. Del. 1987), *aff'd*, 865 F.2d 1247, 9 U.S.P.Q.2d 1461 (Fed. Cir. 1980) (“lack of practical utility cannot co-exist with infringement and commercial success); (“Utility may be evidenced by sales and commercial demand.” (Lipscomb’s Walker on Patents, 5:17, p. 562 (1984)).

Applicant respectfully submits that this is not a case where the sole asserted utility is as an object of use-testing (*See, Brenner v. Manson*, 383 U.S. 519, 148 U.S.P.Q. 689, 696 (1966); “We find absolutely no warrant for the proposition that although Congress intended that no patent be granted on a chemical compound whose sole ‘utility’ consists of its potential role as an object of use-testing, a different set of rules was meant to apply to the process which yielded the unpatentable product”). The dicta in *Brenner* related to the patentability of a chemical compound which itself had no known use. The Court opined that the utility could not solely consist of testing the compound in order to determine a utility for the compound itself. In contrast, the mCAR2 knockout mouse is useful for the study of the utility and function of the mCAR2 gene, and not for the purpose of establishing a utility for the mouse. The practical distinction is clear: one skilled in the art would not understand what to do with a compound without a defined use, but would immediately recognize the use of a knockout mouse having a specific gene disruption.

Finally, Applicant notes that in an Office Action dated November 20, 1993, the Examiner rejected the claimed invention as obvious under section 103. The Examiner argued: “[o]ne of

ordinary skill in the art at the time the invention was made would have been motivated to disrupt the nuclear hormone receptor gene instead of the gene disrupted by Capecchi to determine the function of the nuclear hormone receptor of SEQ ID NO:1 *in vivo*.” (p. 10). Thus, while the Examiner argued that one skilled in the art would have been motivated to make the Applicant’s claimed mouse, he also argues that one skilled in the art would not know how to use such a mouse once created. Applicant submits that the Examiner’s previous statements are an admission that the present invention has a well-established utility, i.e., determining the function of a gene.

Applicant submits that since one of ordinary skill in the art would immediately recognize the utility of a knockout mouse in studying gene function, a utility that is specific, substantial and credible, the invention has a well-established utility, thus satisfying the utility requirement of section 101. On this basis alone, withdrawal of the rejection with respect to the present invention is warranted, and respectfully requested.

In addition, the claimed invention is useful for a particular purpose. The Applicant has demonstrated and disclosed specific phenotypes of the presently claimed mice, i.e., impaired coordination and balance, a spleen abnormality, a thymus abnormality and a lymph node abnormality. Utility of a knockout mouse demonstrating any of these properties would be apparent to, and considered credible by, one of skill in the art.

The Examiner argues that none of the phenotypes correlate to a useful phenotype because the phenotypes described are not specific to a disease and are not linked to a disruption in the human equivalent of SEQ ID No:1.

Applicant respectfully disagrees. The Examiner’s arguments are similar to arguments made by the Patent Office with respect to pharmaceutical compounds the utility of which were based on murine model data, arguments which were dismissed by the Federal Circuit in *In re Brana* (34 U.S.P.Q.2d 1436)(Fed. Cir. 1995). The case involved compounds that were disclosed to be effective as anti-tumor agents and had demonstrated activity against murine lymphocytic leukemias implanted in mice. The court ruled that the PTO had improperly rejected, for lack of utility, claims for pharmaceutical compounds used in cancer treatment in humans, since neither the nature of invention nor evidence proffered by the PTO would cause one of ordinary skill in art to reasonably doubt the asserted utility.

The first basis for the Board's holding of lack of utility (the Board adopted the examiner's reasoning without any additional independent analysis) was that the specification failed to describe any specific disease against which the claimed compounds were useful, and therefore, absent undue experimentation, one of ordinary skill in the art was precluded from using the invention. (*In re Brana* at 1439-40). The Federal Circuit reasoned that the leukemia cell lines were originally derived from lymphocytic leukemias in mice and therefore represented actual specific lymphocytic tumors. The court concluded that the mouse tumor models represented a specific disease against which the claimed compounds were alleged to be effective. (*In re Brana* at 1440).

The Board's second basis was that even if the specification did allege a specific use, the applicants failed to prove that the claimed compounds were useful.

The Federal Circuit responded: “[A] specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as in compliance with the enabling requirement of the first paragraph of Section 112 unless there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support.” (*Brana* at 1441, *citing In re Marzocchi*, 439 F.2d 220, 223, 169 USPQ 367, 369 (CCPA 1971)). From this it followed that the PTO has the initial burden of challenging a presumptively correct assertion of utility in the disclosure. Only after the PTO provides evidence showing that one of ordinary skill in the art would reasonably doubt the asserted utility does the burden shift to the applicant to provide rebuttal evidence sufficient to convince such a person of the invention's asserted utility. (*Id.*)

The court held that the Patent Office had not met its burden. The references cited by the Board did not question the usefulness of any compound as an antitumor agent or provide any other evidence to cause one of skill in the art to question the asserted utility of applicants' compounds. Rather, the references merely discussed the therapeutic predictive value of *in vivo* murine tests -- relevant only if the applicants were required to prove the ultimate value in humans of their asserted utility. The court did not find that the nature of the invention alone would cause one of skill in the art to reasonably doubt the asserted usefulness. The purpose of treating cancer with chemical compounds did not suggest an inherently unbelievable undertaking or involve implausible scientific principles. (*Id.*)

The Court concluded that one skilled in the art would be without basis to reasonably doubt the asserted utility on its face. The PTO had not satisfied its initial burden. Accordingly, the applicants should not have been required to substantiate their presumptively correct disclosure to avoid a rejection under the first paragraph of Section 112. (*Id.*)

As in *Brana*, Applicant has asserted that the claimed invention is useful for a particular practical purpose, an assertion that would be considered credible by a person of ordinary skill in the art. For example, the mCAR2 deficient mice have demonstrated impaired coordination and balance, and abnormalities in the spleen, thymus and lymph nodes. Based on these phenotypes, the mice demonstrate a role for the mCAR2 gene in physiological development. They would be useful in further study in the field of developmental biology. The mice also demonstrate a role for the gene in motor coordination, thus serving a use for further study in the field of neuroscience. It is also possible that these abnormalities in human may be associated with mutations in hCAR. Thus, such mice are useful for studying whether such conditions in humans are associated with the human CAR2 gene and for developing treatment strategies and therapeutics associated with any such mutations. The mice may also be useful in drug development strategies where, for example, they can be used as controls to determine the effectiveness or specificity of putative agents that target the mCAR or hCAR gene or its expression product by comparing the animal's physiological response to that of a wild-type animal.

That the specification does disclose a link between these particular phenotypes and the gene in mice and humans does not detract from the utility of the mice. In *Brana*, the claimed compound had demonstrated activity against a murine tumor implanted in a mouse. Yet, the Federal Circuit found that utility had been demonstrated. Here, the invention relates to a disruption to a murine gene in a mouse. Like the tumor mouse model, the knockout mouse with a specific gene disrupted is a widely accepted model, the utility of which would be readily accepted in the art (see, for example, Austin et al., cited above). It is submitted that one skilled in the art would be without basis to be reasonably doubt Applicant's asserted utility, and therefore the Examiner has not satisfied his initial burden.

It is respectfully submitted that the Examiner needs to assess utility in light of the nature of the invention. Applicant is claiming a knockout mouse. The burden should not be placed on Applicant to establish that hCAR mutations in humans result in the same phenotypes observed in

mice. This task is more appropriately placed on the commercial and academic entities conducting further research using the present invention. As noted by the Federal Circuit, usefulness in patent law necessarily includes the expectation of further research and development. (*In re Brana* at 1442).

In summary, Applicant submits that the claimed mCAR2 knockout mouse, regardless of any disclosed phenotypes, has inherent and well-established utility in the study of the function of the mCAR2 gene, and thus satisfies the utility requirement of section 101. Moreover, Applicant believes that the specific phenotypes of the transgenic mice demonstrate that the mice are useful for a specific practical purpose that would be readily understood by and considered credible by one of ordinary skill in the art.

In light of the arguments set forth above, Applicant does not believe that the Examiner has properly established a *prima facie* showing that establishes that it is more likely than not that a person of ordinary skill in the art would not consider that any utility asserted by the Applicant would be specific and substantial. (*In re Brana*; MPEP § 2107). Withdrawal of the rejections is therefore respectfully requested.

B. Rejections under 35 U.S.C. § 112, 1st para.

Claims 39-52 stand rejected as failing to comply with the written description requirement. The Examiner argues that “mCAR gene” recited in claim 39 is new matter. Claim 39 has been amended to recite “mCAR2.”

The Examiner argues that claim 51 recital of “tissue” is new matter. Claim 51 has been canceled.

Withdrawal of the rejections is respectfully requested.

C. Rejections under 35 U.S.C. § 112, 2nd para.

Claims 39-52 have been rejected as being indefinite. The Examiner argues that the metes and bounds of “mCAR gene” recited in claim 39 cannot be determined. Claim 39 has been amended to recite “mCAR2.” Withdrawal of the rejection is respectfully requested.

It is submitted that the claims are currently in condition for allowance, and notice to that effect is respectfully requested. The Commissioner is hereby authorized to charge any deficiency or credit any overpayment to Deposit Account No. 13-2725.

In view of the above amendments and remarks, Applicant respectfully requests a Notice of Allowance. If the Examiner believes a telephone conference would advance the prosecution of this application, the Examiner is invited to telephone the undersigned at the below-listed telephone number.

Respectfully submitted,

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Date

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The Knockout Mouse Project

Mouse knockout technology provides a powerful means of elucidating gene function *in vivo*, and a publicly available genome-wide collection of mouse knockouts would be significantly enabling for biomedical discovery. To date, published knockouts exist for only about 10% of mouse genes. Furthermore, many of these are limited in utility because they have not been made or phenotyped in standardized ways, and many are not freely available to researchers. It is time to harness new technologies and efficiencies of production to mount a high-throughput international effort to produce and phenotype knockouts for all mouse genes, and place these resources into the public domain.

Now that the human and mouse genome sequences are known^{1–3}, attention has turned to elucidating gene function and identifying gene products that might have therapeutic value. The laboratory mouse (*Mus musculus*) has had a prominent role in the study of human disease mechanisms throughout the rich, 100-year history of classical mouse genetics, exemplified by the lessons learned from naturally occurring mutants such as *agouti*⁴, *reeler*⁵ and *obese*⁶. The large-scale production and analysis of induced genetic mutations in worms, flies, zebrafish and mice have greatly accelerated the understanding of gene function in these organisms. Among the model organisms, the mouse offers particular advantages for the study of human biology and disease: (i) the mouse is a mammal, and its development, body plan, physiology, behavior and diseases have much in common with those of humans; (ii) almost all (99%) mouse genes have homologs in humans; and (iii) the mouse genome supports targeted mutagenesis in specific genes by homologous recombination in embryonic stem (ES) cells, allowing genes to be altered efficiently and precisely.

The ability to disrupt, or knock out, a specific gene in ES cells and mice was developed in the late 1980s (ref. 7), and the use of knockout mice has led to many insights into human biology and disease^{8–11}. Current technology also permits insertion of 'reporter' genes into the knocked-out gene, which can then be used to determine the temporal and spatial

expression pattern of the knocked-out gene in mouse tissues. Such marking of cells by a reporter gene facilitates the identification of new cell types according to their gene expression patterns and allows further characterization of marked tissues and single cells.

Appreciation of the power of mouse genetics to inform the study of mammalian physiology and disease, coupled with the advent of the mouse genome sequence and the ease of producing mutated alleles, has catalyzed public and private sector initiatives to produce mouse mutants on a large scale, with the goal of eventually knocking out a substantial portion of the mouse genome^{12,13}. Large-scale, publicly funded gene-trap programs have been initiated in several countries, with the International Gene Trap Consortium coordinating certain efforts and resources^{14–17}.

Despite these efforts, the total number of knockout mice described in the literature is relatively modest, corresponding to only ~10% of the ~25,000 mouse genes. The curated Mouse Knockout & Mutation Database lists 2,669 unique genes (C. Rathbone, personal communication), the curated Mouse Genome Database lists 2,847 unique genes, and an analysis at Lexicon Genetics identified 2,492 unique genes (B.Z., unpublished data). Most of these knockouts are not readily available to scientists who may want to use them in their research; for example, only 415 unique genes are represented as targeted mutations in the Jackson Laboratory's Induced Mutant Resource database (S. Rockwood, personal communication).

The converging interests of multiple members of the genomics community led to a meeting to discuss the advisability and feasibility of

a dedicated project to produce knockout alleles for all mouse genes and place them into the public domain. The meeting took place from 30 September to 1 October 2003 at the Banbury Conference Center at Cold Spring Harbor Laboratory. The attendees of the meeting are the authors of this paper.

Is a systematic project warranted?

A coordinated project to systematically knock out all mouse genes is likely to be of enormous benefit to the research community, given the demonstrated power of knockout mice to elucidate gene function, the frequency of unpredicted phenotypes in knockout mice, the potential economies of scale in an organized and carefully planned project, and the high cost and lack of availability of knockout mice being made in current efforts. Moreover, implementing such a systematic and comprehensive plan will greatly accelerate the translation of genome sequences into biological insights. Knockout ES cells and mice currently available from the public and private sectors should be incorporated into the genome-wide initiative as much as possible, although some may be need to be produced again if they were made with suboptimal methods (e.g., not including a marker) or if their use is restricted by intellectual property or other constraints. The advantages of such a systematic and coordinated effort include efficient production with reduced costs; uniform use of knockout methods, allowing for more comparability between knockout mice; and ready access to mice, their derivatives and data to all researchers without encumbrance. Solutions to the logistical, organizational and informatics issues associated with producing, characterizing and distributing such a large number of

*The Comprehensive Knockout Mouse Project Consortium**

*Authors and their affiliations are listed at the end of the paper.

COMMENTARY

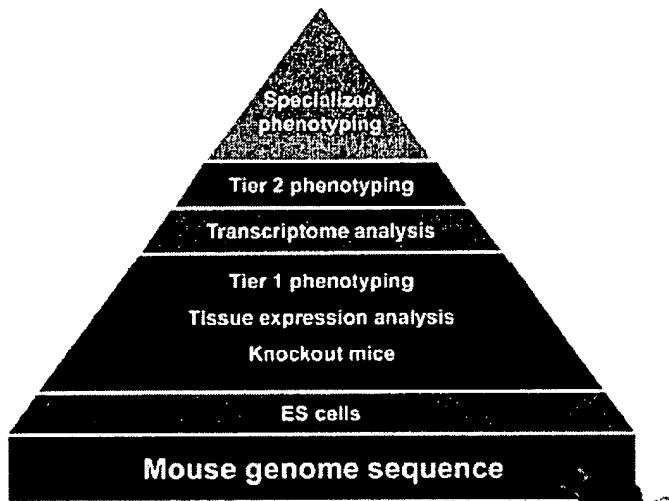


Figure 1 Structure of resource production in the proposed KOMP. Using the mouse genome sequence as a foundation, knockout alleles in ES cells will be produced for all genes. A subset of ES cell knockouts will be used each year to produce knockout mice, determine the expression pattern of the targeted gene in a variety of tissues and carry out screening-level (Tier 1) phenotyping. In a subset of mouse lines, transcriptome analysis and more detailed system-specific (Tier 2) phenotyping will be done. Finally, specialized phenotyping will be done on a smaller number of mouse lines with particularly interesting phenotypes. All stages will occur within the purview of the KOMP except for the specialized phenotyping, which will occur in individual laboratories with particular expertise.

mice will draw from the experience of related projects in the private sector and in academia, which have made or phenotyped hundreds of knockout mice using a variety of techniques. Lessons learned from these projects include the need for redundancy at each step to mitigate pipeline bottlenecks and the need for robust informatics systems to track the production, analysis, maintenance and distribution of thousands of targeting constructs, ES cells and mice.

Null-reporter alleles should be created
The project should generate alleles that are as uniform as possible, to allow efficient production and comparison of mouse phenotypes. The alleles should achieve a balance of utility, flexibility, throughput and cost. A null allele is an indispensable starting point for studying the function of every gene. Inserting a reporter gene (e.g., β -galactosidase or green fluorescent protein) allows a rapid assessment of which cell types normally support the expression of that gene. Therefore, we propose to produce a null-reporter allele for each gene. Making each mutation conditional in nature by adding *cis*-elements (e.g., *loxP* or *FRT* sites) would

be desirable, but we do not advocate this as part of the mutagenesis strategy unless the technological limitations currently associated with generating conditional targeted mutations on a large scale and in a cost-effective manner can be overcome.

A combination of methods should be used
Various methods can be used to create mutated alleles, including gene targeting, gene trapping and RNA interference. Advantages of conventional gene targeting include flexibility in design of alleles, lack of limitation to integration hot spots, reliability for producing complete loss-of-function alleles, ability to produce reporter knock-ins and conditional alleles and ability to target splice variants and alternative promoters. BAC-based targeting has the potential advantages of higher recombination efficiencies and flexibility for producing complex mutated alleles¹⁸. Gene trapping is rapid, is cost-effective and produces a large variety of insertional mutations throughout the genome but can be somewhat less flexible^{17,19-21}. There is uncertainty regarding the percentage of gene traps that produce a true null allele and the fraction

of the genome that can ultimately be covered by gene-trap mutations. Trapping is not entirely random but shows preference for larger transcription units and genes more highly expressed in ES cells. In recent studies, gene trapping was estimated to potentially produce null alleles for 50–60% of all genes, perhaps more if a variety of gene-trap vectors with different insertion characteristics is used^{17,21}. RNA interference offers enormous promise for analysis of gene function in mice²² but is not yet sufficiently developed for large-scale production of gene modifications capable of reliably producing true null alleles. Both gene-targeting and gene-trapping methods are suitable for producing large numbers of knockout alleles, and, given their complementary advantages, a combination of these methods should be used to produce the genome-wide collection of null-reporter alleles most efficiently.

What should the deliverables be?

A genome-wide knockout mouse project could deliver to the research community a trove of valuable reagents and data, including targeting and trapping constructs and vectors, mutant ES cell lines, live mice, frozen sperm, frozen embryos, phenotypic data at a variety of levels and detail, and a database with data visualization and mining tools. At a minimum, we believe that a comprehensive genome-wide resource of mutant ES cell lines from an inbred strain, each with a different gene knocked out, should be produced and made available to the community. Choosing an inbred line (129/SvEvTac or C57BL/6), and evaluating the alternative of using F_1 ES cells and tetraploid aggregation to provide potential time savings, merits additional scientific review and discussion^{23,24}. ES cells should be converted into mice at a rate consistent with project funding and the ability of the worldwide scientific community to analyze them. Although the value and cost-effectiveness of systematically characterizing the mice is a matter of debate, a limited set of broad and cost-effective screens, probably including assessment of developmental lethality, physical examination, basic blood tests, and histochemical analysis of reporter gene expression, would be useful. More detailed phenotyping, based on findings from the initial screen or existing knowledge of the gene's function, could be done at specialized centers. All ES cell clones and mice (as frozen embryos or sperm) should be available to any researcher at minimal cost, and all mouse phenotyping and reporter expression data should be deposited into a public database.

In determining how to implement the project, utility to the research community should be the standard for judging value. Each step after ES cell generation (e.g., mouse creation, breeding, expression analysis, phenotyping) will make the resource useful to more researchers but will also increase costs and scientific complexity. We therefore advocate a 'pyramid' structure for the project (Fig. 1). At the base of the pyramid is the genome-wide collection of mutant ES cells for every mouse gene. Over time, a subset of these mutant ES cells should be made into mice and characterized with an initial phenotype screen (Tier 1; Fig. 1) and analysis of tissue reporter-gene expression. A subset of these lines should be profiled by microarray analysis, and a subset of these profiled by system-specific (Tier 2) phenotyping, based on the results of the Tier 1 phenotyping, array studies, existing knowledge of the gene's function and the gene's tissue expression pattern. With time, the upper tiers of the pyramid will be filled out, eventually transforming the pyramid into a cube, with information of all types available for all genes.

This project will require the resolution of numerous intellectual property claims involving the production and use of knockout mice. To deal with the existing patents that cover the technologies and processes involved in the production of mutant mice, we suggest that a 'patent pool', such as that used in the semiconductor industry²⁵, should be generated. Several individuals who represent entities that control patents on mouse knockout technologies are authors on this paper, and they agree with this approach. We also agree that any mutant ES cells or mice produced should be placed immediately in the public domain.

Mechanisms and costs

ES cell production. Automated knockout construct and ES cell production should be carried out in coordinated centers to ensure efficiency and uniformity. We estimate that most known mouse genes could be knocked out in ES cells within 5 years, using a combination of gene-trapping and gene-targeting techniques. Gene trapping can produce a large number of mutated alleles quickly, but its progress should be monitored closely to determine when its yield of new genes diminishes¹⁷ and, therefore, when targeting should be increasingly relied on. As large-scale trapping projects have already defined gene classes that probably cannot be knocked out by trapping (e.g., single-exon GPCRs genes that are not expressed in ES cells), we propose that targeting begin on those classes immediately. All ES cells should be made available to the research community, because this collection itself

would be a valuable resource. Efforts in the public and private sectors have already knocked out many genes in ES cells, and, to the degree that the alleles produced fit the prescribed characteristics (i.e., null alleles with a reporter) and are available, every effort should be made to incorporate these into the planned public resource. Costs for generating this part of the resource were estimated at between \$9–11 million/year for five years (these and all subsequent figures are direct costs).

Mouse production. The subset of ES cells made into mice each year should be chosen by a peer-review process. Central facilities for high-efficiency mouse production, genotyping, breeding, maintenance and archiving should be funded, to take advantage of efficiencies of scale in mouse creation and distribution. Researchers could apply to produce groups of mice outside the centers, as long as they meet the cost specifications of the program. All mice should be made available immediately to researchers as frozen embryos or sperm, for nominal distribution cost. An initial target of 500 new mouse lines per year would double the current rate at which new genes are knocked out in the public sector; we feel that this rate is within the capacity of the biomedical research community worldwide to absorb and analyze. We estimated the initial cost of this level of mouse production to be \$12.5–15 million per year.

Reporter tissue expression analysis. Approximately 30 tissues from adult and developmental stages should be sampled to cover the main organ systems. Analysis methods should be customized to the organ system and marker, and a searchable database of the sites of gene expression, and the images showing them, should be produced. Centers to carry out these analyses and data curation should be selected by peer review. We estimated the cost of this component for 500 mouse lines to be \$2.5–5 million per year, depending on how much tissue sectioning and cell-level analysis is done.

Phenotyping. Tier 1 phenotyping should be a low-cost screen for clear phenotypes and should be done on all mouse lines produced. Tier 1 should include home-cage observation, physical examination, blood hematological and chemistry profiles, and skeletal radiographs. The centers producing the mice should carry out the Tier 1 analyses, at an estimated cost of \$2.5 million per year for 500 lines. Selected lines, chosen on the basis of findings from Tier 1 phenotyping, tissue expression patterns, microarray data and the scientific literature, should undergo more detailed and system-focused Tier 2 phenotyping. Tier 2 phenotyping should be done in

specialized phenotyping centers, akin to those already in operation for phenotyping of mice produced by ENU mutagenesis. All Tier 1 and Tier 2 phenotyping should be done on a uniform genetic background by dedicated groups of individuals in single locations, to facilitate consistency and cross-comparison of results among different mouse lines. All Tier 1 and Tier 2 phenotyping results should be deposited into a central project database freely accessible to the research community. More detailed and specialized phenotyping could be done by individual researchers in their own laboratories; deposition of this more detailed phenotype data would be encouraged.

Transcriptome analysis. Transcriptome profiling of tissues from each knockout line, collected in a uniform way across all mice and tissues and placed into a searchable relational database, would add substantially to the scientific value of the project, though it would also add considerably to its cost. Transcriptome analysis should therefore be done on a subset of mice, chosen by peer review. We estimate that, with the best currently available array technology, an analysis of ten tissues would cost ~\$18,000 per line.

Conclusions

This project, tentatively named the Knockout Mouse Project (KOMP), will be a crucial step in harnessing the power of the genome to drive biomedical discovery. By creating a publicly available resource of knockout mice and phenotypic data, KOMP will knock down barriers for biologists to use mouse genetics in their research. The scientific consensus that we achieved—that a dedicated project should be undertaken to produce mutant mice for all genes and place them into the public domain—is important but is only the beginning. Implementation of these recommendations will require additional input from the greater scientific community, including those responsible for programmatic direction and financial support of biomedical research in the public and private sectors. This ambitious and historic initiative must be carried out as a collaborative effort of the worldwide scientific community, so that all can contribute their skills, and all can benefit. International discussions among scientific and programmatic staffs since the Banbury meeting at Cold Spring Harbor, in both the public and private sectors, have shown that there is great enthusiasm and commitment to this vision. The next step for KOMP will be to move this visionary plan from conceptualization to implementation, with an urgency befitting the benefits it will bring to science and medicine.

COMMENTARY

URLs. The curated Mouse Knockout & Mutation Database is available at <http://research.bmnn.com/mkmdb>. The curated Mouse Genome Database is available at <http://www.informatics.jax.org/>. Patent pool: A solution to the problem of access in biotechnology patents? is available at <http://www.uspto.gov/web/offices/pac/dapp/opsa/patentpool.pdf>.

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